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THE MOSAIC DISEASE OF TOBACCO.[1]

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INTRODUCTION.

"Mosaic" is one of the most serious and widespread diseases known to affect the tobacco plant. This disease also is known locally as "calico," "gray-top," "mottled-top," "mottling," and "foxy" tobacco. Especially in southern tobacco sections the term "frenching"[2] is used to designate abnormal, sickly plants characterized by the development of stringy, very thick, and leathery leaves which may be free from mottling. It has not been determined whether this is distinct from mosaic or only a somewhat different phase of this disease. In the tobacco sections of Kentucky and Tennessee "wal-loon" is often used to denote mosaic or other abnormal appearances more or less closely resembling symptoms of the mosaic disease. The disease has also been termed "chlorosis." Finally, mosaic plants sometimes are spoken of as "brindle" or "mongrel." These terms

[1] In a study of the mosaic disease of tobacco facts have been learned which throw considerable light on its nature and origin. It has been shown that it is communicable to plants of many genera of the solanaceous family and that aphides are sometimes active disseminators of infection both in the greenhouse and in the field. The fact that particular insects are involved in the spread of the disease not only helps to explain the contradictory results obtained by previous investigators, but also serves to indicate means by which the disease can, to a greater or less extent, be controlled under practical field conditions.

Since insects are involved in the spread of the mosaic disease both in seed beds and in the field and the practical control of this disease must depend primarily upon an intimate knowledge of the habits and distribution of species which may act as capable carriers of infection, this phase of the investigation has been taken up in cooperation with the Bureau of Entomology.—Wm. A. Taylor, *Chief of Bureau.*

[2] The term "frenching" or "frenched" is applied to sickly, spindling corn plants and peach trees as well as to tobacco plants. Its origin dates back to the settlement of Maryland when the English settlers applied the term "Frenchmen" to weak, spindling tobacco plants as a term of contempt. See Clayton, John. A Letter . . . to the Royal Society, May 12, 1688, giving an account of several observables in Virginia . . . p. 19. (*In* Force, Peter. Tracts and Other Papers, Relating Principally to the Origin, Settlement, and Progress of the Colonies in North America, v. 3, Washington, 1844.)

are usually descriptive of the characteristic mottling or otherwise sickly appearance of plants affected with the disease. It is true, however, that they are in many instances somewhat loosely applied to a variety of abnormal appearances and symptoms not at all associated with the specific mosaic disease of tobacco.

Both in Europe and America the mosaic disease of tobacco has been the subject of wide inquiry. Hitherto no investigator has been able to offer very conclusive evidence which would consistently explain the baffling nature and mysterious origin of the disease. Each in his own way, however, has emphasized a favorite opinion, so that the literature of the disease is especially conspicuous for its widely contrasted theories.

The view seems to be generally accepted that the disease is a physiological or functional disorder, although it has long been known that it is more or less infectious. For this reason the writer's experiments were at first planned along physiological lines. Facts soon came to light, however, which led to the conclusion that the disease must be parasitic in its origin rather than physiological or functional.

The writer has found that particular aphides can induce mosaic symptoms in plants infested by them, and much evidence has accumulated to show that aphides are responsible for serious outbreaks of the disease both in the greenhouse and in the field. Since it has been found that insects may act as carriers of the disease, this phase of the problem has been taken up in cooperation with the Bureau of Entomology with the hope that further light may be thrown on this point.

APPEARANCE OF AFFECTED PLANTS.

The development of the mosaic disease of tobacco is attended with various physiological and morphological changes in the leaves, branches, and sometimes the flowers of all affected plants. The character and intensity of these symptoms vary greatly, depending upon the age, habits of growth, species of plants affected, and external conditions. The following symptoms are more or less characteristic of different phases of the disease at one time or another:

(1) Partial or complete chlorosis.
(2) Curling of the leaves.
(3) Dwarfing and distortion of the leaves.
(4) Blistered or "savoyed" appearance of the leaves.
(5) Mottling of the leaves with different shades of green.
(6) Dwarfing of the entire plant.
(7) Dwarfing and distortion of the blossoms.
(8) Blotched or bleached corollas (in *Nicotiana tabacum* only).
(9) Mosaic sucker growths.
(10) Death of tissues (sometimes very marked in *Nicotiana rustica*).

The extent and character of the first observable symptoms of the disease depend upon the age and vigor of the plants at the time of

inoculation with the virus. In order to study this phase of the question thoroughly the writer has inoculated tobacco plants at all stages of growth from the cotyledon stage until the plants have matured seed.

INITIAL SYMPTOMS IN YOUNG PLANTS.

In very young plants at the two-leaved or three-leaved stage and in much larger plants in the rosette stage the first visible symptom of the mosaic disease usually appears as a slight downward curling and distortion of the smallest, innermost leaves, which at the same time become more or less chlorotic.

As these leaves increase in size, small, abnormally dark-green spots and areas quite generally make their appearance. If the plants are given plenty of room and an abundance of plant food to stimulate active growth, these dark-green portions of the leaves develop rapidly into large, irregular, crumpled swellings, or blisters, more or less characteristic of the so-called "savoyed appearance." At this period these leaves very frequently grow to a size disproportionate to the rest of the plant, becoming long, narrow, sinuous, or otherwise enlarged, distorted, and irregular. On the other hand, if the young plants affected with the disease are badly crowded and starved in the seed bed, growth proceeds very slowly, so that the leaves may show only a slight mottling. Under such unfavorable conditions of growth tiny plants showing on the leaves the dark-green spots characteristic of the true mosaic disease may remain unchanged in size and appearance for months.

Although young plants sometimes appear to outgrow the marked distortion and deformity affecting the immature leaves at this age, the mottled phase of the disease makes its appearance and persists with more or less intensity until all leaf development has finally ceased. (Pl. I, fig. 1, *B*.)

DEVELOPMENT IN PLANTS APPROACHING MATURITY.

Should tobacco plants approaching maturity become infected with the mosaic disease, a characteristic phase known as "mottled-top" or "gray-top" develops. (Pl. I, fig. 2.) Although the number of leaves affected and the intensity of the markings is considerably increased if the disease develops at a comparatively early period in the life of the plant, attenuated mottling in one or two of the small topmost leaves is the only evidence of the malady in plants which develop the disease just prior to final maturity. In many experimental tests made by the writer upon greenhouse plants, typical phases of "mottled-top" have almost invariably appeared whenever the mosaic virus has been introduced into healthy plants just as the small, immature flower head was beginning to emerge and to elon-

gate above the uppermost leaves. At this time the cluster of flower heads is very small, compact, and less than 1 inch across. After an incubation period of 10 to 15 days, the first symptoms of the disease become manifest as a very noticeable wilting of the upper leaves, especially in a hot sun. These leaves, especially in transmitted sunlight, show a very fine mottling, which very rapidly develops into characteristic "mottled-top."

If the leaves are examined closely, it will be seen that this appearance is due to the distribution of the dark-green shades along the fine anastomosing veins, while the lighter shades occupy the small inclosed areas. The earlier manifestations of "mottled-top" in some respects simulate certain color changes which accompany the normal ripening of fully matured leaves. The "mottled-top" phase of the disease in nearly mature plants produces little or no distortion in the leaves which exhibit this appearance. Practical growers do not consider this phase particularly serious.

If plants become infected just prior to the appearance of the blossoms, the leaves of the main stalk, all of which at this time have practically completed growth, are little, if at all, changed in normal appearance. The characteristic symptoms, however, such as distortion, etc., are reproduced in the secondary growths (suckers) in much the same manner that young plants are affected.

ROOTS OF DISEASED PLANTS.

The roots of mosaic plants superficially appear quite as normal in many instances as those of healthy plants. It is probable, however, that the roots as well as the leaves may be more or less seriously impaired in form and function, depending upon the age and condition of the plant when affected with the disease.

LEAVES OF DISEASED PLANTS.

The development of the mosaic disease in tobacco plants produces a great variety of changes in the normal color and form of the leaves. The more evident changes are confined to the actively growing portions, such as the immature leaves of young plants and the vigorous secondary growth of more mature plants. A characteristic feature of the true mosaic disease, which seems to distinguish it from nearly all unhealthy appearances of purely physiological origin, is the distinctive blotched and mottled appearance of the leaves, sometimes accompanied by distortion, especially if the leaves are immature. In very immature plants the leaves often show marked distortion and irregularities of growth, from which they may almost recover as they become larger and more mature.

Fantastic leaf forms frequently arise in mosaic plants. In some instances the development of the lamina is almost entirely suppressed,

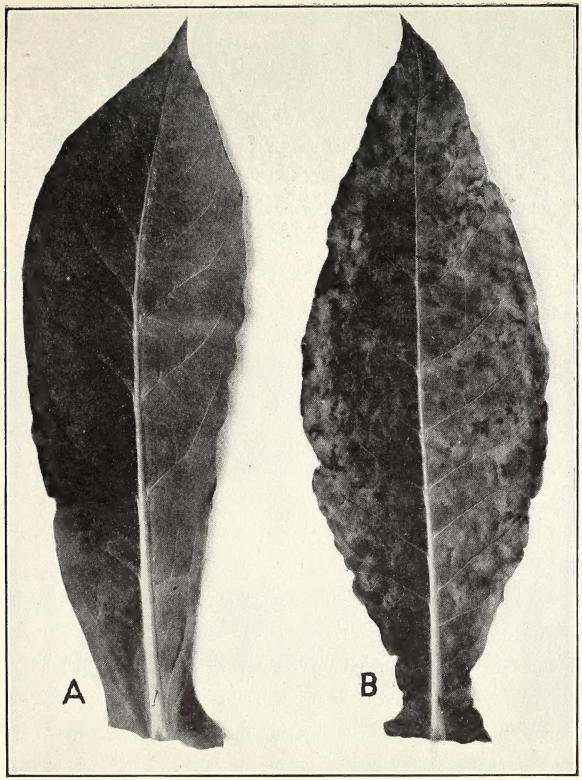

FIG. 1.—HEALTHY AND MOSAIC LEAVES OF NICOTIANA TABACUM. A, HEALTHY LEAF; B, MOSAIC LEAF, SHOWING FINE MOTTLING.

(Greatly reduced.)

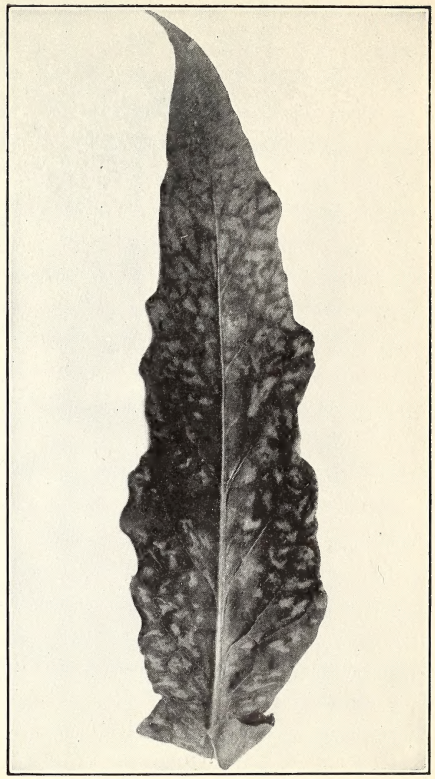

FIG. 2.—TOP LEAF OF NICOTIANA TABACUM, SHOWING THE SO-CALLED "MOTTLED-TOP" PHASE OF THE MOSAIC DISEASE.

The surface irregularities do not appear.
(About four-fifths natural size.)

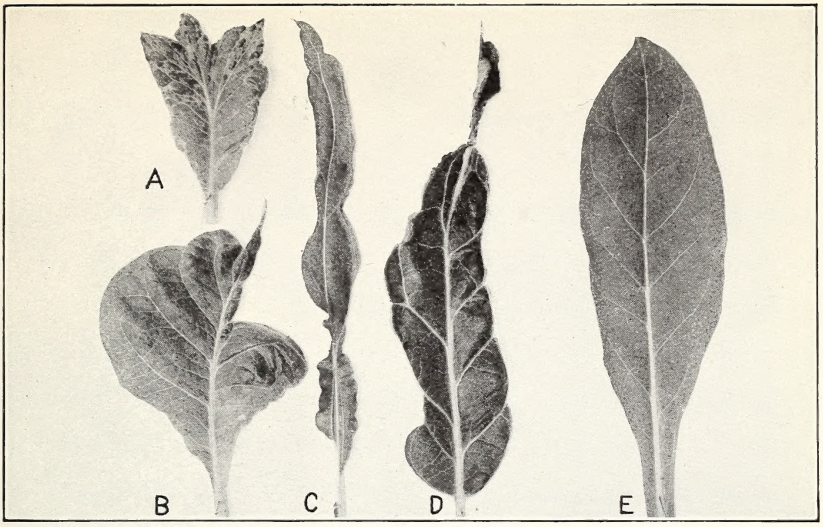

FIG. 1.—MALFORMED MOSAIC LEAVES OF NICOTIANA TABACUM (A, B, C, AND D) AND A HEALTHY LEAF (E).

(Somewhat reduced.)

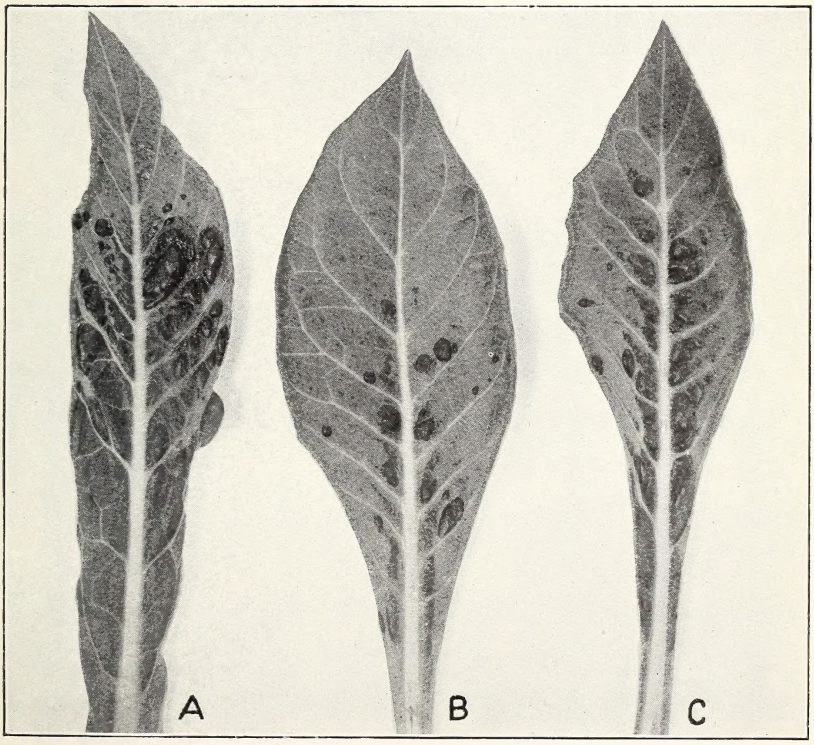

FIG. 2.—MOSAIC LEAVES OF NICOTIANA TABACUM, SHOWING THE DEVELOPMENT OF PROMINENT BLISTERLIKE OUTGROWTHS OF THE LEAF TISSUES.

These swellings appear on the upper face of the leaf and are usually very dark green in color in comparison with the rest of the leaf. (Natural size.)

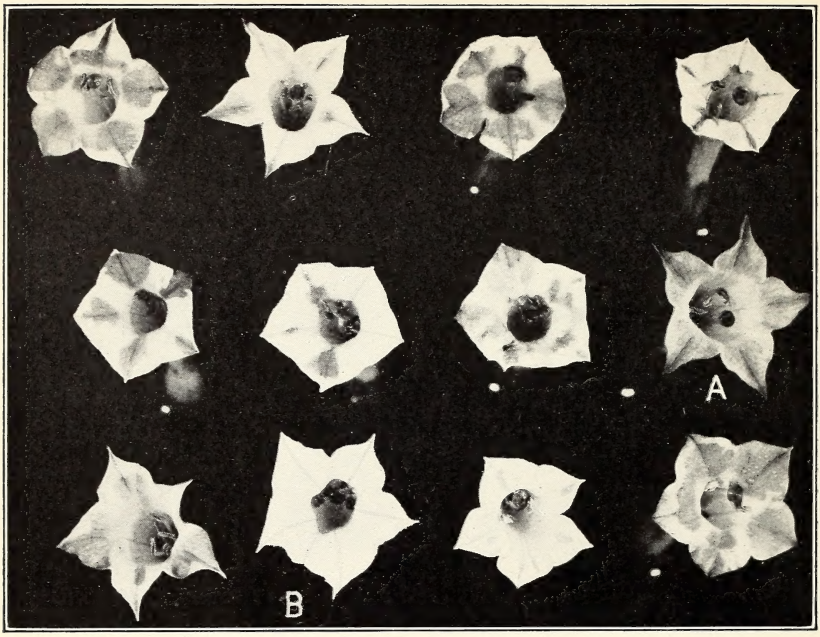

FIG. 1.—COROLLAS OF AMERICAN VARIETIES OF NICOTIANA TABACUM, SHOWING VARIOUS DEGREES OF MOTTLING PRODUCED BY THE MOSAIC DISEASE.

The dark areas represent the normal pink color; the light or white areas indicate a partial or complete absence of red. A, Normal pink blossom; B, mosaic blossom which is entirely white. (About natural size.)

FIG. 2.—BUDS OF NICOTIANA TABACUM, SHOWING DISTORTED AND OTHER DEPAUPERATE BUDS PRODUCED BY THE MOSAIC DISEASE (A) AND NORMAL, HEALTHY BUDS (B).

(About natural size.)

so that the leaf is little more than a twisted midrib. In other instances a long, sinuous, ribbonlike leaf is produced by the growth of a very narrow and irregular leaf blade (Pl. II, fig. 1, *C*.) A very peculiar and irregular leaf shape sometimes arise when the lamina fails to develop beyond certain abnormally thickened veins, which become the margins of the leaf. (Pl. II, fig. 1, *D*.)

A very distinct and irregular phase of mottling is usually produced in immature leaves of young plants by the development of abnormally dark-green swellings, or blisters. These surface prominences may be so small as to appear like mere dots, or so large and confluent as to involve the greater portion of the leaf, and they sometimes occur in definite lines along the midrib or lateral veins of a leaf. Other leaves may appear perfectly normal in color, except for a few dots scattered irregularly here and there. (Pl. II, fig. 2, *A*, *B*, and *C*.)

In later manifestations of the disease the immature top leaves and suckers become beautifully mottled; hence, the term "mottled-top." This phase of mottling is typically developed in upper leaves which have almost completed their growth and is not generally associated with the development of surface swellings, or blisters.

Individual plants inoculated at the same age and in identically the same manner with the same virus frequently exhibit markedly dissimilar phases of the disease. In one plant the disease is of a particularly malignant type, greatly dwarfing and distorting all the leaves; in another plant the symptoms may be shown only as a slight mottling of the leaves throughout the growth of the plant.

BLOSSOMS OF DISEASED PLANTS.

The presence of the mosaic disease in all varieties of *Nicotiana tabacum* usually affects the intensity and distribution of the pink coloration of the blossoms in much the same manner that it affects the intensity and distribution of the green coloration of the leaves. For this reason an examination of the blossoms affords one of the surest indications of the presence of the disease in a tobacco plant. So far as the writer is aware, this distinctive appearance has not before been mentioned. As a rule, in blossoms of mosaic plants the normal pink coloration is present only in lines, specks, or conspicuous blotches. In most instances these markings are very irregular in distribution, sometimes involving a portion or all of the lobes of the corolla. (Pl. III, fig. 1.)

A rather striking and symmetrical color pattern is sometimes afforded by a blossom in which the normal pink coloration occurs only as a fine line in the sinus of each corolla lobe. Other affected plants produce blossoms all of which are devoid of color, so that they appear quite white or very pale. More rarely, the presence of

the disease in a plant produces in the blossoms a pink-purple color several shades darker than that which occurs in normal blossoms of a healthy plant.

The development of the mosaic disease in tobacco plants is not invariably indicated in the blossoms by all the color changes previously described. With regard to the typically blotched appearance of the blossoms, however, the writer has failed to find an instance when this appearance was not associated with the disease in a plant. Experiments with healthy tobacco plants grown in greenhouses at Arlington, Va., during the winter of 1912, indicate, however, that high temperatures together with high humidity may produce very pale or almost white blossoms, indistinguishable from the white or pale blossoms sometimes produced by mosaic plants.

Now and then tobacco plants show the most pronounced symptoms of the disease in the upper leaves and suckers and at the same time produce blossoms entirely normal in color. At other times the disease appears on a single sucker, all the blossoms of which are distinctly mottled, although the blossoms of the main flower head and other suckers are perfectly normal in color. In other plants the blossoms of the main stalk are mottled, while the suckers at the same time produce normal pink blossoms. Even in the same flower head some blossoms may show the most pronounced phases of mottling, while others retain the uniform pink coloration of a healthy blossom. In local manifestations of the disease the immature leaves as well as the blossoms of affected branches usually indicate the disease, although this is not an invariable rule.

By carefully timed inoculation the writer has caused the disease to develop in healthy plants subsequent to the apperance of the first open blossoms. Blossoms appearing in advance of the earliest symptoms of the disease displayed the uniform pink coloration of the normal bloom, while later blossoms becoming affected by the disease displayed the characteristic blotched colorations. The first observable symptoms have sometimes made their appearance in the blossoms alone, and not until suckers had developed was the disease manifest elsewhere in the plant.

In particularly malignant phases of the mosaic disease the plants often produce depauperate and misshapen blossoms. In such instances the corollas remain small and imperfectly formed, so that the distorted, immature stigma and stamens extrude long before the blossom has approached normal maturity. Likewise, on such plants the seed pods are abnormally small and shrunken and may contain very few viable seeds. (Pl. III, fig. 2; Pl. IV, fig. 1.)

The irregular distribution of color in the blossoms of plants affected with the mosaic disease seem to be confined entirely to the pink-flowered varieties of *Nicotiana tabacum*. None of the greenish,

purplish yellow, or white-flowered species of Nicotiana observed by the writer have shown this phenomenon in diseased plants. A few of the more common species observed in this connection may be mentioned here:

Nicotiana silvestris (blossoms white).
N. rustica (blossoms greenish yellow).
N. longiflora (blossoms purplish).
N. alata (blossoms white).
N. langsdorfii (white and yellow flowered forms).
N. paniculata (blossoms greenish).

The red-flowered forms of *Nicotiana forgetiana* have not been modified by the disease.

The various manifestations in the intensity and unequal distribution of color in the blossoms of mosaic tobacco plants bear a striking resemblance to the mottled color of peaches produced by trees affected with peach yellows. Speaking of the mottled color of peaches produced by diseased trees, Smith[1] says:

> The amount of color appears to depend somewhat upon variety. Sometimes there is comparatively little crimson spotting, and again, it is a very marked feature, the skin being almost purple and the flesh of the deepest crimson, even in pure white varieties. In an experience covering four years and including a great many thousand trees diseased by yellows, I have never known but one in which there was entire absence of red spotting in the fruit. This tree bore premature insipid peaches and the characteristic shoots.

Farther on he says:

> In many cases the red-spotted, prematurely ripened fruits are the first indications of disease, or at least the first symptoms striking enough to attract general attention. They are very often borne exclusively upon one or two limbs of otherwise healthy-looking trees. These limbs are not different in appearance from the rest of the tree. They bear vigorous shoots and full-grown, smooth, dark-green foliage; often, also, green, half-grown fruits, which afterwards ripen in a normal manner. There is no indication of disease except in the fruit, which, in color and size, contrasts strikingly with the fine green foliage and the normal, immature fruit.

LOCAL AND GENERAL MANIFESTATIONS OF THE DISEASE.

It is a matter of common observation that obvious symptoms of the mosaic disease do not necessarily appear in all parts of a plant simultaneously. In an affected plant individual leaves, branches, or blossoms of the same flower cluster may show localized symptoms of the disease. A leaf may appear quite healthy and normal except for a few small isolated blisters. Branches healthy in appearance sometimes grow close beside those showing the most pronounced symptoms. Likewise, in a flower head, blossoms normal in color are often intermingled with those showing extreme phases of mottling.

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[1] Smith, Erwin F. Additional evidence of the communicability of peach yellows and peach rosette. U. S. Department of Agriculture, Division of Vegetable Pathology, Bulletin 1, 1891, p. 12–13.

In the "mottled-top" or "gray-top" phase of the disease the topmost growing leaves and suckers first manifest the disease. Leaves lower down on the main stalk usually do not show symptoms of the disease, since these completed their growth in advance of its development.

This phenomenon, coupled with the fact that the disease in young plants affects successively younger and later leaves as they appear on the elongating main stalk, has led to the erroneous belief that the course of the disease is upward and never downward.

By inoculating large, nearly mature plants at various points the writer has brought out some very interesting details concerning the occurrence of local and general manifestations. If the plants are inoculated while young or at a period much in advance of maturity, the mosaic disease in tobacco plants very rarely manifests itself as a local trouble, since practically all suckers, which appear at a considerably later period, become affected. On the other hand, large, well-nourished plants at maturity usually produce an abundance of long, leafy, vigorous suckers. Properly topping the plants at this period stimulates a yet more vigorous sucker development. By introducing the virus into the top of one of these, very pronounced local symptoms of the disease sometimes precede the general phase which invariably follows.

Several weeks prior to blooming, when the immature flower heads were only one-half inch to 1 inch across and suckers were only feebly developed, several series of plants were inoculated with mosaic virus. One series was inoculated by puncturing the main stalk deeply with a long needle just above ground. A second series was inoculated by puncturing the main stalk deeply at several points, beginning at the ground and extending along about two-thirds of its length. A third group of plants was inoculated at the very apex of the immature flower head of the main stalk. Other healthy plants were allowed to mature fully in order to obtain long, vigorous suckers, which, in many instances, arose near the base of the plants and nearly or quite equaled the main stalk in length. One of these was then inoculated with mosaic virus at or near the apex of the immature buds.

When the virus was introduced into the main stalk at its base or at several points along its length all immature parts of the plants, including the top leaves and suckers, usually developed pronounced symptoms of mosaic simultaneously. When the virus was introduced at the very apex of the immature flower heads of the main stalk a pronounced mottling of the corollas of the main flower head in several instances was the only noticeable evidence of the disease, although at this time long vigorous suckers were not infrequently present. Later, however, these also showed the usual disease symptoms. In one plant the writer, by antiseptic methods, carefully cut

away the main stalk, showing only the mottled corollas. Two large, vigorous, apparently healthy lateral suckers several feet in length were left undisturbed, and in three days these also had developed all the symptoms typical of the disease.

Local manifestations of the disease were readily induced in a number of mature plants by inoculating the apex of a tall, vigorous sucker. The earliest symptoms were frequently confined to the branch inoculated and usually first appeared in the small secondary suckers nearest the point of inoculation. Plate IV, figure 2, illustrates a typical instance of this sort. A tall plant of the Sumatra variety was allowed to blossom, and it soon developed suckers freely. As shown in the illustration, some of these were nearly as long as the main stalk. On February 9 one of these (at *A*) was inoculated thoroughly in the immature flower head (when only one-fourth inch across) and at the first bald sucker just below. At this time the flower head *B* of the main stalk had been in bloom several weeks and all the blossoms produced were of a uniform pink color. The branch or sucker (at *C*) had not yet blossomed. On February 21 the first symptoms of the disease appeared in the inoculated branch *A*, the blossoms of which were pale white or conspicuously mottled. Likewise, the small axillary shoots (at *a* and *b*) were just showing mosaic symptoms. No other indications of the disease were noticeable in the plant. Examined again on March 4 the blossoms of the branch *A* were distinctly mottled, although those of the main flower head *B* and the branch *C* were still normal in color. On March 5 a large immature basal sucker was showing mosaic symptoms, and on March 25 this basal sucker was producing leaves and blossoms mottled to a very pronounced degree. The main stalk *B* and the branch *C* did not at any time show symptoms of the disease, either in the small axillary suckers or in the color of the blossoms.

Although local mosaic symptoms may sometimes develop as the result of certain methods of inoculation, the disease invariably becomes general, appearing in other immature growing parts of affected plants. In all respects this progressive development of mosaic is strikingly similar to the behavior of peach yellows, as described by Smith.[1] He says:

> There is also a progressive development of the disease, more and more of the tree becoming involved. In other words, it appears first to be a local disease; then, later on, a general one. Quite often, the first season, only one limb or a few limbs are diseased, but additional limbs are invariably affected the second year. I know of no exception to this. The first year of attack it is comparatively infrequent to find a tree diseased in all its parts; the second year it is somewhat rare to find many sound branches; often there are none.

[1] Smith, Erwin F. Peach yellows, a preliminary report. U. S. Department of Agriculture, Botanical Division, Section of Vegetable Pathology, Bulletin 9, p. 91.

SUSCEPTIBILITY OF SOLANACEOUS PLANTS TO THE DISEASE.

Although Mayer[1] established many important facts concerning the mosaic disease, he states that he did not succeed in communicating the disease from tobacco to other solanaceous plants. Westerdijk[2] has also reported that the mosaic disease of tobacco is distinct from the mosaic disease of tomato. By actual experiment Clinton[3] first proved conclusively that the mosaic disease of tobacco was communicable to healthy tomato plants, and vice versa. The writer's experiments indicate that the two diseases are identical and likewise communicable to the same plants throughout the solanaceous family. The writer has readily transferred the disease from tobacco to a great variety of solanaceous plants. By inoculation the disease has been obtained in the following genera:

In Nicotiana (all varieties of *N. tabacum* tested and many distinct species of Nicotiana).
In Lycopersicon (several of the more distinct varieties of tomato).
In Petunia (*Petunia violacea*).
In Physalis (two distinct garden species).
In Datura (*D. stramonium* and *D. tatula*).
In Hyoscyamus (*H. niger*).
In Solanum (*S. nigrum* and *S. carolinense*).
In Capsicum (several of the more distinct varieties).

Both in the greenhouse and in the field natural agencies produce the disease in practically all the plants mentioned.

A mosaic plant of the species *Solanum carolinense* brought to the writer's attention indicates that the mosaic disease of tobacco sometimes occurs in strictly wild plants.

In the genera Solanum and Atropa, although the methods used to inoculate these plants were particularly severe, all efforts to inoculate the common potato (*Solanum tuberosum*), the eggplant (*Solanum melongena*), and belladonna (*Atropa belladonna*) have failed to produce any visible evidence of the disease.

In the genus Datura some species appear to be considerably more resistant than others to the mosaic disease of tobacco. The disease may be communicated to *D. stramonium* L., *D. tatula* L., and *D. quercifolia* H. B. and K. The most persistent and rigorous methods of inoculation have failed to produce any symptoms of mosaic in the species *D. fastuosa* L.

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[1] Mayer, Adolf. Ueber die Mosaikkrankheit des Tabaks. Die Landwirtschaftlichen Versuchs-Stationen, Bd. 38, p. 450–467, pl. 3, 1886. Abstract in Journal of Mycology, v. 7, no. 4, p. 382–385, 1894.

[2] Westerdijk, Johanna. Die Mosaikkrankheit der Tomaten. Amsterdam, 1910, 19 p., 3 pl. (Mededeelingen uit het Phytopathologisch Laboratorium "Wille Commelin Scholten," Amsterdam, 1.)

[3] Clinton, G. P. Notes on fungous diseases, etc., for 1908. Connecticut Agricultural Experiment Station, Biennial Report, 1907/1908, p. 857–858, pl. 66, fig. 6, 1909.

Evidence at hand indicates that the species *Datura metalloides* L. and *D. leichhardtii* F. Muell. may also be as resistant to the disease as the species *D. fastuosa*. During the season of 1912 the occurrence of the mosaic disease was noted in a plat of Datura species growing at Arlington, Va. The relative susceptibility of these species to infection was well illustrated in this plat. Late in the season practically all the plants of the species *D. stramonium* and *D. quercifolia* had developed well-marked symptoms. On the other hand, the species *D. fastuosa*, *D. metalloides*, and *D. leichhardtii* showed no observable symptoms of mosaic throughout the season.

All efforts to communicate the mosaic disease of tobacco to plants of other families than the Solanaceæ have always given negative results. The following plants have been inoculated by the most rigorous methods without any visible symptoms of the disease:

Polygonaceæ—Sheep sorrel (*Rumex acetosella* L.).
Leguminosæ—
White clover (*Trifolium repens* L.).
Red clover (*Trifolium pratense* L.).
Peanut (*Arachis hypogea* L.)
Soy bean (*Glycine hispida* Moench.).
Cruciferæ—
Cauliflower (*Brassica oleracea* L.).
Peppergrass (*Lepidium* sp.).
Plantaginaceæ—Rib-grass (*Plantago lanceolota* L.).
Labiateæ—Dead nettle (*Lamium amplexicaule* L.).
Oxalidaceæ—Wood sorrel (*Oxalis stricta* L.).
Compositæ—Garden lettuce (*Lactuca sativa* L.).
Phytolaccaceæ—Pokeweed (*Phytolacca decandra* L.)

SPECIES AND SPECIES HYBRIDS OF NICOTIANA.

A considerable number of distinct species of Nicotiana have been inoculated at all stages of growth with the virus of the mosaic disease. Of the various species tested all, with the exception of two or three, readily became affected with the disease. It has been produced in the following species: *Nicotiana silvestris*, *N. rustica*, *N. longiflora*, *N. alata*, *N. plumbaginifolia*, *N. forgetiana*, *N. paniculata*, and *N. langsdorfii*.

On the other hand, after the most persistent and rigorous methods of inoculation, the species *Nicotiana glauca* and *N. viscosum*, so far as observable symptoms are concerned, do not seem to be affected by the disease. *N. langsdorfii*, which appeared to be immune, finally developed the disease in one or two plants after a long series of inoculations with the virus. Plants of this species when inoculated through the stalk and petioles seem particularly susceptible to a very destructive and progressive rot, which begins at the point of inoculation and finally kills the plant by slowly involving the surround-

ing tissues. *N. viscosum* is sometimes killed in exactly the same manner, yet no attendant mosaic symptoms have ever appeared in the affected plants. Although frequent inoculations have been made deep into the tissues of all parts of *N. glauca* and *N. tabacum*, these species have never shown any indications of rot at the punctured points. Plants of the species *N. glauca* and *N. viscosum* have been repeatedly cut back and mosaic virus poured upon all cut surfaces. This severe treatment has invariably failed to produce any indications of the disease.

Other experiments were carefully made to determine whether the sap of mosaic plants could be rendered innocuous by mixing it with different proportions of the sap of immune species. At first a number of trials were carried out by mixing equal quantities of mosaic virus and the sap of *Nicotiana viscosum*. As controls, the sap of healthy plants was substituted for the mosaic virus and diluted in the same manner. The mixtures were shaken thoroughly and allowed to stand several hours. Later trials were made in exactly the same manner, using the sap of the immune species of *N. glauca*. In passing, it may be remarked that the diluted virus in no instance lost its infectious properties, since every plant developed the disease in 15 to 18 days following inoculation. All controls remained healthy.

The virus was still further diluted with the sap of *Nicotiana glauca* and tested as follows: Eighteen young plants of Connecticut Broadleaf were arranged in two series of nine plants each. One series was inoculated with a mixture of one-tenth mosaic virus and nine-tenths healthy glauca sap. This was shaken and allowed to stand three hours at a greenhouse temperature of 80° to 85° F. before using. Nine control plants were inoculated in the same manner, using healthy tobacco sap diluted with glauca sap in the same proportions. Needle inoculations in the leaves were made on December 22. Examined on January 9, eight of the series inoculated with the diluted virus had developed the mosaic disease. All the controls were healthy. It is evident that the virus was not rendered innocuous.

In further tests with immune species the writer has made many successful graftings of susceptible varieties of *Nicotiana tabacum* and *N. rustica* with the immune species *N. glauca* and *N. viscosum*. The scions in both instances by subsequent inoculations readily became diseased, but the immune stocks, which were constantly putting forth vigorous branches, have never shown indications of the disease, although these were growing permanently and intimately in contact with the badly diseased scion. Many inoculation experiments have shown conclusively that the virus of the mosaic scions is likewise present in the leaves and shoots of the *N. glauca* stock, although no visible mosaic symptoms are produced. Furthermore,

FIG. 1.—NORMAL AND MOSAIC BLOSSOMS OF NICOTIANA TABACUM.

Top row, normal blossoms. Bottom row, blossoms from mosaic plants showing an extremely depauperate growth of the corollas, so that the stamens and pistils extrude. (Greatly reduced.)

FIG. 2.—SUMATRA TOBACCO PLANT WHICH WAS INOCULATED (AT A) WITH THE MOSAIC DISEASE.

The first symptoms of the mosaic disease appeared at *a* and *b*. The disease became general at a later period and large basal suckers showed pronounced symptoms of it.

FIG. 1.—SCION OF NICOTIANA GLAUCA (A) WHICH HAS BEEN GRAFTED UPON A MOSAIC PLANT OF NICOTIANA RUSTICA (B).

Although the stock is badly diseased the graft shows no visible symptoms. (Reduced.)

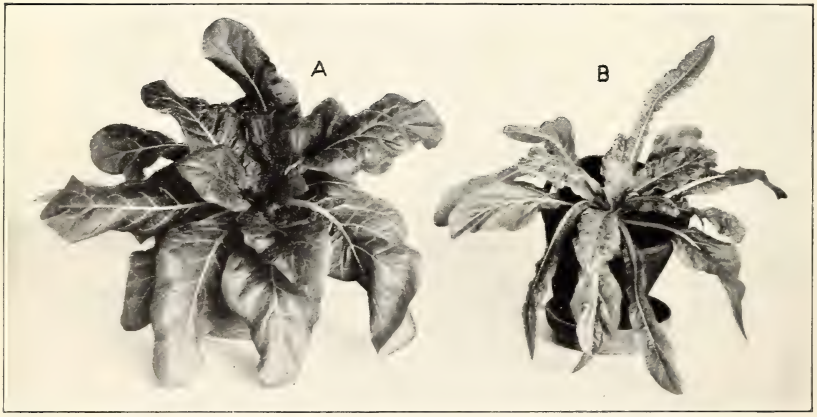

FIG. 2.—HEALTHY AND MOSAIC PLANTS OF NICOTIANA LONGIFLORA. A, HEALTHY PLANT; B, MOSAIC PLANT PRODUCED BY ARTIFICIAL INOCULATION.

(Greatly reduced.)

the mosaic disease can be produced in healthy scions by inoculating the virus into the tissues of *N. glauca*. Plate V, figure 1, shows a scion of *N. glauca* growing upon a badly diseased stock of *N. rustica*.

As to the virulent nature of the sap of the *Nicotiana glauca* stock bearing the mosaic scion of *N. tabacum*, the following experiments afford conclusive evidence. On February 28 three groups of 25 to 30 small plants of the species *N. silvestris* were inoculated as follows: One group, as a control, was inoculated with the sap of a healthy *N. glauca* plant. A second group was inoculated with the sap of a stock of *N. glauca* upon which a mosaic scion of *N. tabacum* was growing. A third group was inoculated with the juice of the mosaic scion itself. On March 11 the control series was perfectly healthy, but a considerable number of mosaic plants had appeared in the other two groups. On March 21 all the plants in the control lot were healthy, while in the remaining two groups practically every plant had become infected with the mosaic disease. Throughout the experiment these plants were carefully screened in a cage so as to exclude all insects.

The above experiment was repeated as follows: Thirteen young Sumatra plants were inoculated with sap from the same stock of *Nicotiana glauca*, and 10 other plants were inoculated with the sap of a healthy plant of *N. glauca* to serve as controls. On April 1, 10 plants of the 13 had developed the disease. Every plant of the control series remained healthy. These experiments were repeated several times at later dates and further confirmed the results of earlier experiments that the sap of the *N. glauca* scion carried the mosaic virus.

Hybrids of several distinct species of Nicotiana also have been obtained and grown in order to determine their immunity to the mosaic disease during the first generation. It was not possible to carry the work beyond plants of the first generation, since in every instance the plants were absolutely sterile. With a single exception these hybrids have not shown the least degree of immunity. In truth, owing to their exceptionally rapid and vigorous growth, some of these hybrids have proved to be especially good material for the study of the disease in all its phases. The following species hybrids have been made and tested:

Nicotiana tabacum ♀ × *N. silvestris* ♂.
N. longiflora ♀ × *N. alata* ♂.
N. tabacum ♀ × *N. glauca* ♂.
N. tabacum ♀ × *N. viscosum* ♂.

Owing to its extremely rapid and uniform growth, its numerous leaves, and the readiness with which it succumbs to the mosaic disease, the writer has used the hybrid *Nicotiana tabacum* ♀ × *N. silvestris* ♂ very extensively in the experimental study of the disease. The hybrid *N. tabacum* ♀ × *N. glauca* ♂ also readily succumbs to the disease,

although it has been impossible to produce observable mosaic symptoms in the male parent.

After many trials the writer finally secured an abundance of viable seed from a single capsule of *Nicotiana tabacum* fertilized with pollen of *N. viscosum*. This hybrid, in size of plant, in leaf and blossom characters, etc., is an intermediate form between the two parents. The most vigorous and persistent methods of inoculating the mosaic virus into plants of this hybrid have invariably failed to produce observable symptoms of the disease.

These hybrids, like the male parent, *N. viscosum*, are attacked by a very destructive rot at the points of injury if the mosaic virus is punctured into the stems.

MOSAIC DISEASE IN DIFFERENT SPECIES OF NICOTIANA.

In the genus Nicotiana the degree of expression of particular mosaic symptoms depends to a considerable extent upon the species affected with the disease.

All pink-flowered varieties of Nicotiana usually indicate the presence of the disease by a more or less conspicuously blotched appearance of the corollas in addition to various foliage symptoms. So far as the writer's observations extend, no other plants manifest the disease in this characteristic manner.

The mottled phase of the mosaic disease is particularly pronounced in plants of the species *Nicotiana longiflora*, *N. silvestris*, and *N. plumbaginifolia*. (Pl. V, fig. 2, and Pl. VI, fig. 1.) This distinctive appearance of the foliage in its extreme form occurs in *N. longiflora*. Likewise, in affected plants of this species the tendency to produce abnormally long, sinuous, ribbonlike leaves is especially marked. In mosaic plants of the species *N. longiflora* and *N. silvestris*, as a result of abnormal division in the region of the apical cells, bifurcated leaves not infrequently arise. These forms have not been observed in any varieties of *N. tabacum* affected with the disease.

Nicotiana rustica (from seed known as the D'Amersfort) by experimental inoculation readily becomes affected with the mosaic disease. In plants of this species the development of the disease is particularly malignant, oftentimes in young plants completely dwarfing the plants and inhibiting practically all foliage growth and flower formation. (Pl. VII.) The mottled phase is less evident in mosaic plants of this species than in any other species observed. The "savoyed" appearance of the leaves, however, is an especially striking feature of mosaic in plants of this species. Directly or indirectly, as a result of the disease many affected plants are frequently attacked by a progressive decay of the tissues, which sometimes involves the entire plant, killing it to the ground.

In affected plants of the species *Nicotiana paniculata* the corolla tube of the small greenish flowers frequently becomes bent or crumpled in the middle.

VARIETIES AND VARIETAL CROSSES OF NICOTIANA TABACUM.

In connection with various inoculation experiments with the mosaic disease, practically all the distinct varieties of *Nicotiana tabacum* and crosses among them have been used. None of them has shown immunity to the disease. The rapidity of development of the disease, the intensity of the mosaic markings of the leaves of affected plants, etc., are more or less dependent upon the variety, its vigor, the size and abundance of the leaves, and their shade of green. The most malignant symptoms of the disease are frequently associated with the more distinctive White Burley variety. In affected plants of this variety badly depauperate and bleached corollas and an intensely chlorotic appearance of all the leaves are of common occurrence.

THE MOSAIC OF TOBACCO PROBABLY DISTINCT FROM POKEWEED MOSAIC.

It is a fact of considerable interest to note that pokeweed (*Phytolacca decandra*) is attacked by a mosaic disease which produces symptoms in every way similar to those produced by the mosiac disease in tobacco plants. Although the mosaic disease of pokeweed is highly infectious to healthy pokeweed plants, all experiments to date confirm the statement that the virus of mosaic pokeweed plants does not produce mosaic symptoms in tobacco plants. Likewise the virus of mosaic tobacco plants has never produced the disease in healthy pokeweed plants.

Healthy pokeweed plants have been repeatedly cut back and inoculated for long periods with the virus of mosaic tobacco plants. The same procedure was followed with other pokeweed plants, using the virus of mosaic pokeweed. In the first experiment mosaic symptoms never appeared. The virus of mosaic pokeweed plants, however, produced typical pokeweed mosaic, usually within 15 to 20 days following inoculation.

The following experiments show the results of inoculating different lots of tobacco plants in pots on the same date, some with the virus of mosaic pokeweed plants, others with the mosaic virus from tobacco: On June 13, 15 healthy tobacco plants 6 to 8 inches high were inoculated with tobacco mosaic virus. The first symptoms appeared in practically every plant June 22–23, 9 to 10 days later. On June 13, 28 similar tobacco plants were inoculated with pokeweed mosaic virus. Symptoms of disease had not appeared in these plants which were left undisturbed until July 11. On that date, 14 of these plants

were arranged in 2 rows of 7 each and inoculated with the virus of mosaic tobacco plants. The first mosaic symptoms were showing in these plants on July 23. On July 28, the entire 14 plants showed pronounced mosaic symptoms. With the exception of a single plant, the remaining 14 plants which had been inoculated with pokeweed mosaic on June 13 were still healthy. These plants came into bloom on August 13 and still remained free from the disease, with the exception of one or two plants.

On June 14, 14 similar plants were inoculated with tobacco-mosaic virus. The first mosaic symptoms showed in nearly every plant June 23 and 24, 9 to 10 days later. On June 14, 20 similar plants were inoculated with pokeweed mosaic virus. The disease did not develop in these plants.

On August 13, when all the plants were just coming into blossom, 2 rows of 7 plants each were selected from the plants treated on June 13 and 14 with the virus of mosaic pokeweed. One row of 7 plants was then inoculated with the mosaic virus of tobacco. The remaining row of plants was inoculated with the mosaic virus of pokeweed. On August 26, 13 days after inoculation, 6 of the 7 plants receiving the virus of tobacco mosaic had developed the disease. The entire lot of 7 inoculated with the pokeweed mosaic remained free from the disease as late as October 9, when seed pods were fully ripened.

In October, inoculation experiments were again made with tobacco, using the virus of mosaic pokeweed and of mosaic tobacco plants. On October 10, 20 small Connecticut Broadleaf plants in pots were inoculated with the juice of mosaic pokeweed plants. Using the same mortar for extracting the juice, 20 similar plants were inoculated with the virus of mosaic tobacco. The first mosaic symptoms began to appear in these plants on October 21, 11 days later. On October 27, mosaic symptoms had developed in 17 of the 20 plants treated with the virus of mosaic tobacco. On the other hand, not a single case of the disease appeared in the series of 20 plants treated with pokeweed mosaic, although these plants were kept under observation until February 1, 1913, at which time they had reached complete maturity.

INCUBATION PERIOD OF THE DISEASE.

Following inoculation a rather variable period precedes the appearance of the first visible symptoms. The length of this period depends to a considerable extent upon the kind of plant, the age of the plant when infected, and such external factors as may retard or accelerate growth.

So far as the writer has been able to learn, eight days, reported by Woods,[1] is the shortest period previously observed between the time

[1] Woods, A. F. Observations on the mosaic disease of tobacco. U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 18, 1902, p. 17.

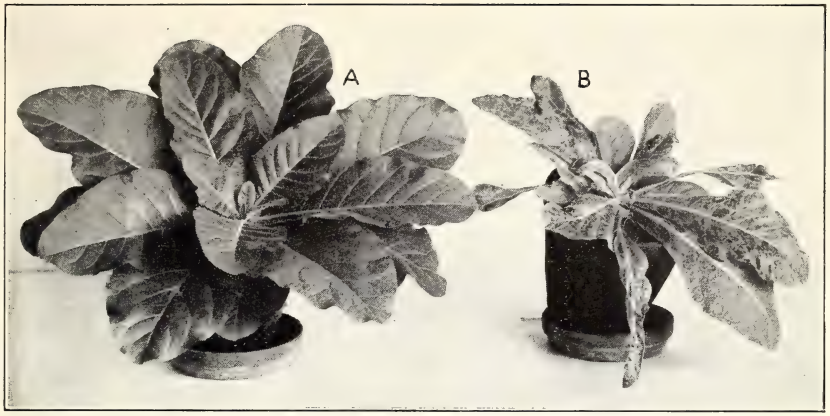

FIG. 1.—HEALTHY AND MOSAIC PLANTS OF NICOTIANA SILVESTRIS. A, HEALTHY PLANT; B, DISEASED PLANT PRODUCED BY ARTIFICIAL INOCULATION.

(Greatly reduced.)

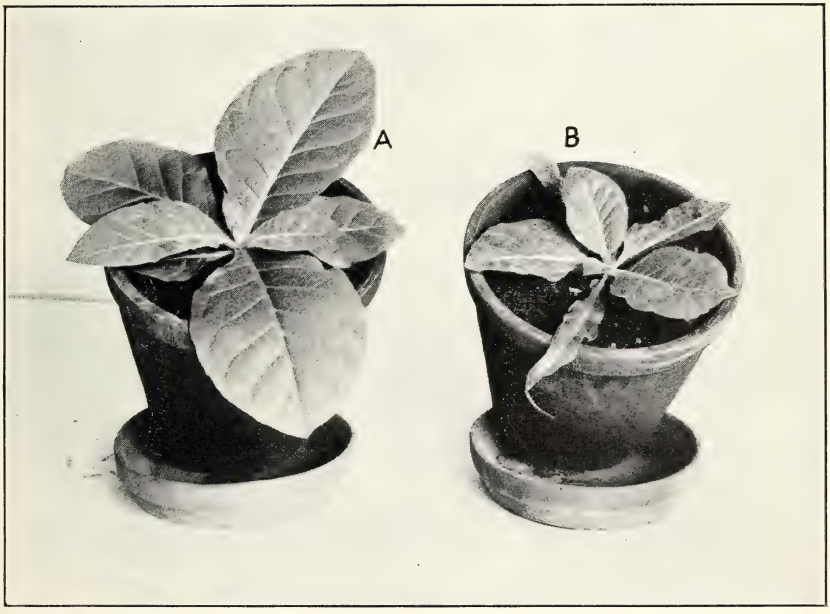

FIG. 2.—HEALTHY AND MOSAIC PLANTS OF NICOTIANA TABACUM. A, HEALTHY PLANT; B, MOSAIC PLANT, SHOWING SYMPTOMS OF THE DISEASE FOLLOWING INFESTATION WITH APHIDES.

Hundreds of mosaic plants were obtained in this manner as a result of aphid infestation. (Greatly reduced.)

HEALTHY AND MOSAIC PLANTS OF NICOTIANA RUSTICA. A, HEALTHY PLANT; B, DISEASED PLANT OF NICOTIANA RUSTICA PRODUCED BY ARTIFICIAL INOCULATION FROM A DISEASED PLANT OF NICOTIANA TABACUM.

(Greatly reduced.)

of inoculation and the appearance of the first mosaic symptoms. Mayer [1] produced the disease in 10 to 12 days. In the writer's experiments the shortest period elapsing between inoculation and the expression of the first visible symptoms was six to seven days. In experiments during the years 1912 and 1913 many hundreds of tobacco plants were inoculated with the mosaic virus at all stages of growth, from the appearance of the cotyledons to complete maturity. The results of some of these inoculations are as follows:

Ten good-sized plants of several American varieties of tobacco inoculated on November 18, 1912. First symptoms noted in 16 to 17 days.

Five plants of *Nicotiana tabacum* ♀ × *N. glauca* ♂ inoculated on December 8, 1912. First symptoms noted in 14 to 15 days.

One hundred good-sized plants of *N. tabacum* ♀ × *N. silvestris* ♂ inoculated on December 8, 1912. First symptoms appeared in 10 to 11 days.

Four young plants of *N. tabacum* ♀ × *N. glauca* ♂ inoculated on December 12, 1912. First symptoms noted in 12 days.

Six rather large plants inoculated on December 16, 1912. First symptoms appeared in 20 days.

Twelve plants of *N. tabacum* ♀ × *N. silvestris* ♂ inoculated on December 16, 1912. First symptoms noted in 20 days.

Nine Broadleaf plants inoculated on December 21, 1912. First symptoms appeared in about 15 days.

Nine young plants of Connecticut Broadleaf inoculated on December 26, 1912. First symptoms appeared in 11 days.

Twelve full-sized, nearly mature tobacco plants inoculated on February 8, 1912. First symptoms noted in 12 to 15 days.

Sixty-six small tobacco plants inoculated on May 8, 1912. First symptoms were showing in nearly every plant on May 14 to 15, i. e., 6 to 7 days following inoculation.

Eight plants of *Nicotiana paniculata* inoculated May 10, 1912, showed first observable symptoms 20 days later.

Twenty-nine young tobacco plants inoculated on June 14, 1912, showed first observable symptoms in practically all within 9 to 10 days.

Seventy young, vigorous tobacco plants inoculated on March 28, 1912, showed first observable symptoms in 6 to 7 days.

Thirty young plants inoculated on December 9, 1912, showed practically every plant diseased January 10 to 11, about 30 days later. These plants were grown in a very cool greenhouse, so that the rate of growth of the plants was very slow.

Thirty young plants inoculated on January 4, 1913, showed first symptoms 18 to 20 days later.

Six or seven days probably is about the minimum period required to show the first visible mosaic symptoms in tobacco at whatever stage of growth the plants are inoculated. The disease manifests visible symptoms usually after 12 to 15 days. Low temperature conditions that generally retard growth appear indirectly to retard the visible expression of symptoms, which are usually associated with actively growing cells and tissues.

[1] Mayer, Adolf. Op. cit.

Numerous experiments have shown that the disease develops very readily in 10 or 12 days in large, vigorous plants inoculated just prior to blossoming or even after all blossoms have ceased to appear in the flower head of the main stalk. The progress of the disease is then coincident with the development of suckers at one point or another on the plant.

DISTRIBUTION OF VIRUS IN DISEASED PLANTS.

As already pointed out, obvious symptoms of the mosaic disease do not necessarily appear simultaneously in all the leaves and branches of an affected plant. If nearly mature plants become affected with the disease, the topmost leaves and suckers usually develop unmistakable symptoms, while the lowermost mature leaves always remain perfectly normal in appearance. In many instances a single sucker at the base of a plant becomes typically diseased, while at the same time others growing beside it show no symptoms whatever. Although it has been proved experimentally many times that the sap of mosaic leaves contains an infectious virus, it has not been definitely shown that this virus may reside in all parts of the affected plants.

Many inoculation experiments made by the writer have shown conclusively that the apparently healthy lower leaves, the roots, and even the corollas of mosaic plants contain the virus of the disease.

On December 26, 1911, 27 young, healthy Connecticut Broadleaf plants growing in 4-inch pots were arranged in 3 series of 9 each. One series was inoculated with the sap of the apparently healthy lower leaves of a plant indicating mosaic disease in only the small leaves at the top. The second series was inoculated with the sap of the topmost mosaic leaves of this same plant. The third series, which served as a control, was inoculated with the sap of a healthy plant. The inoculations in each plant of the three series were made in identically the same manner by puncturing the leaf blade and midrib of one or two leaves. When examined on January 6, 12 days after inoculation, all 9 plants of the series treated with the sap of the topmost mosaic leaves had developed the disease. Of the 9 plants in the series inoculated with the sap of the apparently healthy lower leaves of the same plant 6 had developed the disease. The 9 controls were perfectly healthy. When examined about a week later each series remained as before.

On January 5 this experiment was repeated, using 4 series of 8 or 9 plants each. All plants were inoculated alike at two points by placing the virus upon the stub of a leaf which had been cut off and by puncturing the leaf blade of a second leaf, as follows:

Nine plants were inoculated with the sap of the mosaic topmost leaves of a diseased plant.

Eight plants were inoculated with the sap of the apparently healthy lowermost leaves of a diseased plant.

Eight plants were inoculated with the sap of the roots of a diseased plant.
Nine plants were inoculated with the sap of a healthy plant.

When examined 16 days later 8 out of 9 plants in the series inoculated with the mosaic topmost leaves of the diseased plant were badly diseased. Of the 8 plants inoculated with the sap of the apparently healthy lowermost leaves of this plant, 5 had also developed the disease. Of the 8 plants inoculated with the juice of the roots, 6 plants had become diseased. The 9 controls treated with the juice of a healthy plant remained healthy.

Inoculations made with the juice of corollas of mosaic plants were carried out in the following manner: Three series of 9 plants each were arranged for the experiment. These plants were grown in 4-inch pots and were of the same age, size, and variety as those used in the two preceding tests. The plants of each series were inoculated alike on June 11 by puncturing two or three leaf blades.

Nine plants were inoculated with the sap of mosaic leaves of a diseased plant.
Nine plants were inoculated with the sap of the corollas of this same plant.
Nine plants were inoculated with the sap of a healthy plant, as a control.

In 11 days (June 22) all of the 9 plants inoculated with juice expressed from corollas of the mosaic plant had become diseased; likewise all of the 9 plants of the series treated with the mosaic leaves of this same plant were diseased, but the 9 controls receiving the healthy juice remained healthy.

Similar results were again obtained in a second experiment. On March 28, 20 young Sumatra plants growing in small pots were arranged in two rows of 10 each. One series of 10 plants was inoculated with the expressed juice of corollas of a mosaic plant; the second group of 10, serving as controls, was inoculated in the same manner with the expressed juice of corollas of a healthy plant. On April 15, 6 of the 10 plants receiving the sap from corollas of a mosaic plant had developed the malady. The 10 controls remained free from disease.

Various similar experiments have always confirmed these results. It is evident at once that an active infectious principle resides in all parts of mosaic plants, although such plants frequently indicate only local symptoms of the disease.

DISSEMINATION OF VIRUS FROM THE POINT OF INOCULATION.

If the mosaic virus is pricked into a leaf of a healthy tobacco plant, an infectious principle disseminates itself from this point into all parts of the plant. The following experiment gives some idea of the rate of dissemination of the virus from the point of inoculation:

Seventy young, vigorous Connecticut Broadleaf plants growing in pots were arranged in 7 rows of 10 plants each. On March 28, 1912,

two leaves of every plant were inoculated in exactly the same manner by injecting the virus into each leaf at two points, one on each side of the midrib. These punctures were made toward the apex of the leaf, about two-thirds the distance from the base to the tip.

By grasping each leaf near the apex and snapping it off quickly at the point where the petiole joins the stem, the two inoculated leaves were carefully removed from a row of 10 plants on each successive day beginning with March 29. Using this method of detaching the leaves, accidental infection could not be conveyed by the hand to the exposed surface of the severed petiole.

By removing these two inoculated leaves on successive days the following results were obtained:

Row 1. Two leaves removed March 29; all plants remained healthy.
Row 2. Two leaves removed March 30; all plants remained healthy.
Row 3. Two leaves removed March 31; all plants remained healthy.
Row 4. Two leaves removed April 1; two plants only developed the disease.
Row 5. Two leaves removed April 2; eight plants became diseased.
Row 6. Two leaves removed April 3; eight plants became diseased. The symptoms began showing in some of these plants on April 3, the day the leaves were removed.
Row 7. The inoculated leaves of row 7 were not removed, since eight of the plants were already diseased on April 4.

The results in row 3 indicate that the virus had not passed into the stem from the inoculated leaves which were removed three days after inoculation, since none of these plants developed the disease. Allowing the inoculated leaves to remain on the plant four days from inoculation in some instances gave the virus time to pass into the stem. All removals later than this failed to prevent the development of the disease in the plants, showing that the virus had already passed from the leaves into the main stem.

Experiments have shown that the sap obtained from infected plants between the time of inoculation and prior to the appearance of the first observable symptoms will produce the disease in healthy plants.

During the writer's experiments the disease developed throughout a series of controls following inoculation with the sap of a supposedly healthy plant. Since this plant itself very soon developed symptoms, it is clear that the sap having been obtained during the incubation period of the disease in this plant contained the mosaic virus, the disease coming into expression later.

DURATION OF THE DISEASE IN AFFECTED PLANTS.

As the result of their observations of the mosaic disease, Beijerinck and Woods have mentioned that plants under certain conditions sometimes appeared to recover from the disease.[1] Lode-

[1] Some practical growers have claimed that the disease can be checked if taken at its first appearance by pulling affected plants until they are loosened from the soil. There is little in this view, however, to recommend its general adoption.

wijks[1] claims to have effected a complete cure by treatment with blue light. The young, mosaic portions of the treated plants were covered, while at the same time the lower apparently healthy leaves were subjected to blue light. He states that the blue light stimulated the healthy leaves to produce an antivirus which destroys or renders inert the virus of the disease. Lodewijks also stated that red light decreased the disease and that suffused light checked it. If the malady in question was true infectious mosaic disease, one is inclined to believe that covering the young plants temporarily reduced the color contrasts of the mottled areas. These changes may have led Lodewijks to conclude that a partial or a complete cure had been effected in his experiments.

In the writer's experimental tests many thousands of affected plants have been kept under observation throughout all phases of the disease. In no instance has there been a case of actual recovery from true mosaic disease. Independent of all external conditions, the disease remains more or less in evidence so long as the plant continues to grow. Likewise the writer has never known a plant of *Nicotiana tabacum* to die prematurely as a direct result of the disease. Conditions favorable or unfavorable to vigorous growth affect mosaic and healthy tobacco plants in much the same manner. In fact, the most pronounced mosaic symptoms develop only in well-nourished, succulent, rapidly growing plants, which at maturity also are capable of supporting a vigorous secondary (sucker) growth.

In a seed-bed experiment mosaic plants which had become affected when very small, even though growing in a poor soil, badly crowded, and insufficiently watered, at the end of five months were still maintaining themselves and elongating slowly in spite of the injurious effects of the disease. Several of the more vigorous plants had outstripped their fellows by a few inches and were preparing to put forth some blossoms. Other tiny plants were completely hidden beneath the thickly crowded leaves of the more vigorous individuals. The leaves of these stunted and overcrowded plants affected with the disease, although distinctly blotched with dark-green areas, were little, if at all, "blistered" or "savoyed." Under conditions forming rapid and vigorous growth these dark-green areas usually produce pronounced surface swellings.

No curative treatment has been found for the mosaic disease of tobacco. Gile,[2] working with pineapple chlorosis in Porto Rico, showed that this condition was completely overcome so long as

[1] Lodewijks, J. A., jr. Zur Mosaikkrankheit des Tabaks. Recueil des Travaux Néerlandais, v. 7, p. 107–129. Abstract in Botanisches Centralblatt, Bd. 114, No. 20, p. 518, 1910.

[2] Gile, P. L. Relation of calcareous soils to pineapple chlorosis. Porto Rico Agricultural Experiment Station, Bulletin 11, p. 33, 2 pl., 1911.

solutions of iron salts were applied to the leaves. Likewise, crystals of ferrous sulphate applied to the roots were effective. In the writer's experiments with tobacco, mosaic leaves in some instances have been painted with a 1 to 2 per cent solution of ferrous sulphate. This solution in different strengths has been introduced into the leaf tissues and other parts of mosaic plants. In no instance has this treatment produced any noticeable effect in checking the subsequent course of the disease. Keeping mosaic plants in darkness serves to induce marked chlorosis, which merely diminishes to some extent the color contrasts in mottled leaves.

Although it does not appear possible to free a plant from the mosaic disease, the expression of obvious symptoms depends almost entirely upon the treatment which affected plants receive. During the progress of the malady in small, stunted plants the leaves, when young, are sometimes so slightly changed in appearance that many of these finally appear to outgrow all traces of the disease. In such plants this feeble and transitory expression of the disease makes it appear as if there were a gradual migration of obvious symptoms toward the top of the plant.

On the other hand, a rapid and extensive development of symptoms is coincident with rapid growth. Likewise, conditions which retard growth must of necessity check the further appearance of mosaic leaves and branches.

EXCISION EXPERIMENTS WITH DISEASED PLANTS.

Various excision experiments have shown that the complete removal by antiseptic methods of all portions of a plant showing visible mosaic symptoms does not rid the plant of the disease. Pronounced symptoms later appear in other growing portions of plants thus treated. The excision of mosaic branches sometimes seems actually to hasten the appearance of pronounced symptoms of the disease in branches previously normal in appearance. Plants showing mosaic symptoms only in the topmost leaves or even in the blossoms alone may be cut completely to the ground, yet mosaic symptoms, often of the most malignant character, again appear in the shoots which arise from these stubs.

SOME PROPERTIES OF THE VIRUS OF THE DISEASE.

The sap of mosaic plants is highly infectious to all healthy susceptible plants. An extremely minute quantity of the virus injected into the tissues is capable of initiating the disease. Tests have also shown that simply pouring the sap of mosaic plants liberally upon the leaves of healthy plants is oftentimes sufficient to communicate the disease.

Mayer has shown that the mosaic virus is killed near a temperature of 176° F. Iwanowski[1] first demonstrated that the sap of mosaic plants was still infectious after passing through a Chamberland filter. Beijerinck[2] and Koning[3] later obtained the same results. Beijerinck[4] states that he found the unfiltered juice, volume for volume, more effective than the filtered. The writer has filtered the virus through a Berkfeld filter, but the perfectly clear filtrate remains as highly infectious as the unfiltered sap. Just how long this filtered sap will retain its virulence has not been determined. Tests by the writer show that it was still infectious after being bottled for four months. Beijerinck, also, found the filtered sap infectious after being kept more than three months and mentions that weak solutions of formalin did not kill the virus, while heating to the boiling point did. Beijerinck found that dried mosaic leaves retained their infectious qualities for two years. The writer has found that air-dried mosaic material, ground and bottled, was still highly infectious at the end of 18 months. In fact, inoculation with dried material mixed and macerated with water appears to be the surest method of communicating the disease to healthy plants.

Experiments with fresh mosaic leaves placed in soil in large pots kept out of doors during the winter of 1911–12 seem to indicate that the virus in this condition retained its infectious properties to some extent. It was thought that these leaves would decay, but the continuous, severe freezing weather evidently kept this process in check. Portions of these buried leaves, practically dry and green in color, were ground into a paste with water and pricked into young, healthy plants. The results of inoculations were far less certain than with fresh juice or bottled mosaic material. Healthy leaves left out of doors under the same conditions did not produce the disease at any time.

Numerous experiments have also shown that the mosaic virus preserved in ether, toluene, or glycerin retains its infectious properties for a considerable length of time. The mosaic virus preserved as follows was still infectious to healthy plants on March 5:

10 c. c. virus+1 c. c toluene prepared December 9.

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[1] Iwanowski, D. Ueber zwei Krankheiten der Tabakspflanze. Land- und Forstwirtschaft, 1892. (Russisch.) Abstract in Beihefte, Botanisches Centralblatt, Jahrg. 3, p. 266–268, 1893. (Original not seen.)

[2] Beijerinck, M. W. Ueber ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. Verhandelingen, Koninklijke Akademie van Wetenschappen, Amsterdam, sect. 2, deel 6, no. 5, 22 p. 2 pl., 1898. Abstract in Centralblatt für Bakteriologie [etc.], Abt. 2, Bd. 5, No. 1, p. 27–33, 1899.

[3] Koning, C. J. Die Flecken- oder Mosaikkrankheit des holländischen Tabaks. Zeitschrift für Pflanzenkrankheiten, Bd. 9, Heft 2, p. 65–80, fig. 1–2, pl. 2, 1899.

[4] Beijerinck, M. W. De l'existence d'un principe contagieux vivant fluide, agent de la nielle des feuilles de tabac. Archives Néerlandaises des Sciences Exactes et Naturelles, s. 2, t. 3, livr. 2, p. 164–186, pl. 5–6, 1899.

8 c. c. virus+1 c. c. ether prepared December 9 (ether again added December 26).
5 c. c. virus+20 c. c. glycerin prepared December 9.
8 c. c. virus+3 c. c. toluene prepared January 4.
6 c. c. virus+6 c. c. glycerin prepared January 4.

The original bottled sap used in these preparations and allowed to undergo free fermentation was also infectious on March 5. Earlier experiments have shown that bottled mosaic sap alone, after undergoing fermentation, is able 4 or 5 months later to produce infection. Tests with fermented bottled virus kept for 15 months did not produce infection.

It is evident that the active causative agents in the sap of mosaic plants show a high degree of resistance to the ordinary destructive agencies.

ROOT INOCULATION WITH THE DISEASE.

The writer's experiments show conclusively that the mosaic disease may be communicated to healthy plants by direct inoculation of the virus into the roots. Several methods of root inoculation have been tested. In some instances young plants have been pulled up and their roots dipped into the sap of mosaic plants. The treated plants were then reset. In other instances, the plants were pulled up and their roots dusted with dried finely ground mosaic material before resetting. Other plants when very young were transplanted into healthy soil, and when these plants had become well established the soil was removed from some of the roots and mosaic sap poured upon the broken surfaces from a pipette.

In whatever manner the roots were treated with the virus, the disease usually developed in the plants after 10 or 12 days. The roots of a plant pulled forcibly from the ground are always more or less broken, so that the virus in most instances probably came directly in contact with the broken ends. Root inoculation, however, does not seem to be as certain to produce the mosaic disease in plants as inoculation through the leaf tissues.

SOIL INFECTION WITH THE DISEASE.

All experimental data at hand indicate that the occurrence of the mosaic disease in the seed bed is not so closely associated with soil infection as has been supposed.

The following method has been used to test the effects of germinating and growing tobacco plants in badly infected soil material: Square, tightly made frames of wood 10 or 12 inches deep and without bottoms were set into a carefully prepared, light, friable soil to a depth of 2 to 3 inches. In some of these frames green mosaic

material, consisting of stem and leaves, was finely chopped into the soil and covered with a shallow layer of fine soil. Others, serving as controls, were given the same soil preparation without the addition of plant material. These frames were kept carefully screened until the plants outgrew them. As soon as the young plants appeared fumigations with nicotine were made from time to time to prevent infestation by aphides. The size of each frame, the amount of green mosaic stems and leaves chopped into the soil, and the occurrence of the mosaic disease in each are shown below. The plants were kept under observation at all stages of development until the close of each test.

Frame No. 1, control, 18 inches square; nothing put into the soil; seed sowed August 9. When examined on October 6 the plants were growing vigorously and all were healthy. On December 9 the plants, 36 in number, were 2 feet tall, mostly in bloom, and all healthy but one, which had developed the mosaic disease.

Frame No. 2, 18 inches square; 2,000 grams mosaic stems and leaves chopped into the soil; seed sowed August 9. On October 6 the plants were larger, of a darker green, and more thrifty than the control frame No. 1. They were 12 to 13 inches high and badly crowded, conditions probably due to the fertilizing effect of the decomposed plant material originally added to the soil. When examined on December 9 the 45 plants were 3 to 4 feet high and many were in bloom; all, from the smallest to the largest, were free from disease.

Frame No. 3, control, 18 inches square; nothing added; seed sowed August 9. On October 6 the plants, 30 to 35 in number, were 7 to 8 inches high, dark green, and free from disease. When examined again on December 9 the plants were 2 to 4 feet high, many were in bloom, and all were free from disease.

Frame No. 4, 18 inches square; 3,000 grams of green mosaic stalks and leaves chopped into the soil; seed sowed August 20. On October 6 the plants, although thickly crowded, were 4 to 5 inches high and very green and healthy. Not a single case of mosaic disease was evident. On December 9 the 75 plants averaged 2 to 2½ feet high, growing under badly crowded conditions; many plants were budding. No mosaic disease appeared.

Frame No. 5, 18 inches square; 2,500 grams of mosaic material added to the soil; seed sowed August 20. On October 6 the plants were 3 to 4 inches high and badly crowded; none diseased. On December 9, 30 plants were counted; all were about 2 feet in height and preparing to blossom; none diseased.

Frame No. 6, control, 18 inches square; nothing added to the soil; seed sowed August 20. On October 6 the plants, although crowded badly, were 3 to 4 inches high and free from disease. On December 9 the plants, 45 in number, were about 2 feet high and just ready to bloom; none diseased.

Frame No. 7, 10 inches square; 1,230 grams of green mosaic material added to the soil; seed sowed August 19. On October 6, 40 to 50 plants, averaging 4 to 5 inches high, were growing in this space; badly crowded, yet none diseased. On December 9 there were 35 plants, ranging from 6 inches to 3 feet 8 inches high. The largest plants were in bloom; others were budded; no disease developed.

Frame No. 8, control to frame No. 7, 10 inches square; nothing added; seed sowed August 19. On October 6 the plants were about 3 inches high and all healthy. On December 9 these plants were still free from disease, and their average height was 18 inches, a few reaching the maximum height of 2 feet.

The plants in this frame were considerably smaller than those in frame No. 7, which had received the stems, etc.

In addition to the above tests, which were made under greenhouse conditions, the following were made in the same manner, out of doors, in the experimental tobacco plat:

Frame No. 9, 2 feet square; 2,200 grams of mosaic material chopped into the soil; seed sowed August 4. On October 9 the plants, between 100 and 200 in number, were 10 to 11 inches high; all were healthy but one, which had become diseased and was carefully removed. On October 19 the plants were 12 to 15 inches high. One more plant had become diseased. The rest were fine, healthy plants.

Frame No. 10, control, 2 feet square; seed sowed August 4. On October 9 the plants, several hundred in number, were 10 to 11 inches high and all healthy. On October 19, 2 plants in this control frame were diseased. It is interesting to note here that the mosaic plants in both frames were infested with aphides. Throughout the season these aphides were present on the tobacco plants growing immediately around frames Nos. 9 and 10. Practically all the plants in this field became diseased following infestation. Since the screens protecting these frames were removed on September 27, it is not surprising that these aphides should appear on the young plants.

Many other tests of growing plants in infected soils have been made in addition to these mentioned. Although large quantities of green material have in some instances been added to the soil, this very quickly decomposes and incorporates itself with the soil material. In no instance has an outbreak of mosaic disease appeared in plants germinated and grown in such soils. Plants when young have been pulled and transplanted from infected soils to healthy soils, where they were allowed to blossom, in order to simulate practical methods of handling plants from soils supposedly infected. Out of 20 or 30 plants thus handled not a single plant developed the disease.

ORIGIN OF THE DISEASE DEPENDENT UPON SPECIFIC INFECTION.

Although earlier investigators have concluded that a true infectious mosaic condition can be induced by simply cutting back the plants, the writer's experiments do not support this conclusion.

Many tobacco plants have been used to determine whether the operation of severely cutting them back can in itself produce an infectious mosaic disease. Good-sized individual plants were grown in a screened inclosure, fumigated frequently to prevent infestation by aphides, and cut back at frequent intervals.

Tobacco plant "A," in a screened cage, was cut back on January 31, February 24, March 8, and March 25. This plant continued healthy.

In other experiments tobacco plants were allowed to germinate and to grow thickly crowded in carefully screened cages, which were fumigated from time to time. These plants were cut back at frequent

intervals from the time the plants were 2 or 3 inches high. The operation of cutting back was continued as long as the plants were capable of making growth.

In cage No. 1, 35 plants were cut back on March 8, March 21, March 28, April 10, April 23, May 11, and May 28. These plants remained free from disease.

In cage No. 2, 25 plants were cut back on March 21, March 28, April 10, April 23, May 11, May 28, and June 25. These plants were still free from disease on July 5.

All experiments indicate that plants may be cut back indefinitely if they are free from the mosaic disease at the time of cutting and the infectious principle of the disease is not accidentally introduced during the progress of the experiments. Certain precautions, however, must be very carefully observed. The plants must at all times be kept screened and fumigated to exclude aphides and other insects. All instruments must also be very thoroughly sterilized before cutting back each plant. A flame has afforded the quickest and surest means of sterilizing these instruments.

INSECTS AS CARRIERS OF THE DISEASE.

In the course of the writer's greenhouse experiments with tobacco it was observed that the occurrence of the mosaic disease was associated with aphid infestation. When certain colonies of these aphides were introduced into cages containing healthy plants it was found that the disease usually developed in every infested plant. (Pl. VI, fig. 2.) On the other hand, control tobacco plants never developed the disease if they were grown in screened cages fumigated at short intervals to prevent aphid infestation.

Whether other insects than aphides disseminate mosaic infection to healthy plants yet remains to be determined. This phase of the question has been taken up by the Bureau of Entomology.

During the season of 1912 the occurrence of the mosaic disease in tobacco plants in the seed bed and in the field at Arlington, Va., was unquestionably associated with infestation of the plants by aphides of the species *Macrosiphum tabaci* Perg. A small colony of these insects early established itself at a single point in the seed bed. Somewhat later the disease developed in plants at this point. Shortly after plants from this bed were transplanted to the field one or two sporadic cases of the mosaic disease came into evidence. The lower leaves of these diseased plants were infested with the aphides. These insects sooner or later began to appear beneath the lowermost leaves of practically every healthy plant in the field. At the same time mosaic infection spread rapidly throughout the field until the greater portion of all the plants had developed

the disease. Although these aphides were very common on the under side of the ground leaves during early summer, it was interesting to note that very few could be found a few weeks later.

The occurrence of the mosaic disease in the tobacco seed beds and later in the field at Arlington can not readily be explained on any other grounds than that of transmission by insects. The seed beds were prepared from a good soil, largely mixed with clean sand. This prepared soil was uniform throughout and had never been used before. Nor can the widespread occurrence of the disease throughout the field during the season of 1912 be attributed to mosaic refuse from any previous tobacco crop, since the only crop of tobacco previously produced by this field, at least in recent years, was grown in 1911, and during that season only a very small portion of the field was devoted to tobacco.

WINTER SURVIVAL OF MOSAIC INFECTION.

It would seem to be pretty clearly established that specific infection is wholly responsible for the occurrence of the mosaic disease of tobacco. During an outbreak of the disease an infectious principle in some manner must be carried from plant to plant. Since it has been shown that aphides are sometimes responsible for the spread of the disease, the question arises as to how this infectious principle survives the winter. Whether it can be carried over the winter by hibernating insects yet remains to be determined, but it is hoped that through cooperation with the Bureau of Entomology definite evidence may be obtained on the point.

There is strong evidence in support of the conclusion that the mosaic disease in an affected perennial, such as *Solanum carolinense*, persists throughout the life of the plant. In the roots of such plants the mosaic disease of tobacco doubtless is sometimes carried over the winter. Although the writer has examined many hundreds of plants of *S. carolinense* in various localities, with the exception of the mosaic plant of *S. carolinense* previously referred to, every plant has appeared to be free from the disease. It must be kept in mind, however, that it is frequently very hard to discern external symptoms of the disease in plants of this species. No other susceptible species of the Solanaceæ has shown such slight symptoms of the disease as *S. carolinense*.

It is of some interest here to consider the mosaic disease which affects the perennial pokeweed (*Phytolacca decandra*). The writer has observed that the disease persists from year to year in affected plants of this species. Although all aerial portions of these plants perish during the winter, the infectious principle responsible for the disease is preserved in the underground portions until the following

spring, when the expression of the disease is coincident with the growth of new shoots. On several occasions the writer has observed on these plants the occurrence in large numbers of aphides, which may serve as an important agency in the dissemination of this disease.

OCCURRENCE OF THE DISEASE UNDER PRACTICAL FIELD CONDITIONS.

So far as is known, the mosaic disease of tobacco occurs to a greater or less extent in all countries where tobacco is grown. Mosaic plants may first appear in the seed bed, or at any stage of growth after the plants have been transplanted to the field. The extent of injury caused by the disease depends very largely upon the age of the plants at the time the disease manifests itself.

Not infrequently plants which appear healthy when cut down at maturity give rise to mosaic sucker growths from the stubs throughout a field. This late development and widespread occurrence of the disease might be explained as due to the fact that the instrument used to cut the plants carried infection from mosaic to healthy plants during the process of cutting the stalks. Priming the leaves also would serve to cause a widespread development of the disease in sucker growths appearing very late in the season after the period of harvesting.

The greatest injury is caused when the disease develops in young plants, since these are as a result usually much dwarfed in all their parts. The development of the disease in plants that are nearly mature may in itself result in little or no loss to the grower, on account of normal leaf growth having been already attained. It is a common impression among many growers, however, that mosaic plants are much more susceptible to rust, so that in the end this secondary effect may sometimes occasion great loss.

In the field, the relative distribution of mosaic and healthy plants is sometimes very peculiar. It is claimed that only alternate plants in the rows throughout a field sometimes develop symptoms. It is very evident that field conditions can not account for this peculiar occurrence of the disease. Such uniformity in the distribution of mosaic plants can be explained in only two ways. Since two men riding on a setting machine drop plants alternately in the row, it may be conceived that one man's hands have become badly infected in some way, so that the alternate plants, being the ones which he has dropped, later develop the disease; but it is more reasonable to believe that all the plants in the lot dropped by the one man have been pulled from an infected portion of the seed bed.

Although the opinion seems to be quite generally held that the refuse of mosaic plants plowed into the soil may be responsible for

the disease, there is little evidence to support this view. The fact is well established, however, that the occurrence of mosaic in both the seed bed and the field may bear no relation whatever to a previous outbreak. It has frequently been observed that an epidemic of the disease may occur on new land as well as on land long devoted to tobacco. Both in seed beds and in the field the sporadic occurrence of symptoms is one of the most striking facts connected with the history of the disease. This feature of the disease, together with the fact of its highly infectious nature, has always proved a most serious obstacle confronting all theories which have attempted to account for the mosaic disease on a purely physiological basis.

METHODS OF CONTROL.

While conducting various greenhouse experiments with tobacco during the season of 1911–12, the writer found it almost impossible to grow plants which did not sooner or later become mosaic. At this time it was observed that practically all the plants were infested with aphides and that these insects were responsible for the occurrence of the disease. By careful experiment it was later shown that certain colonies of these insects were the active disseminators of the disease.

In order to secure conditions in every way favorable to the development of mosaic in all unprotected plants, fumigations were withheld until the entire house was overrun with aphides. Large numbers of young tobacco plants were then grown in isolation cages screened with a fine cloth to exclude these insects. At frequent intervals the inclosed plants were also fumigated with nicotine paper. By observing such precautions it proved to be an easy matter to grow plants free from the mosaic disease for any length of time.

Since these preliminary experiments were made, the writer, by keeping the greenhouses thoroughly fumigated against aphides, has grown many thousands of healthy tobacco plants during a period of six months without the occurrence of a single sporadic case of the mosaic disease. Throughout this period mosaic plants were growing on every side in connection with various inoculation studies, yet it was found quite unnecessary to isolate or to screen healthy plants so long as fumigation was continued.

Although all evidence at hand indicates that the mosaic disease can be readily controlled under greenhouse conditions, practical means of preventing its occurrence in the seed bed by similar methods of procedure have not been attempted. This phase of the question is now under investigation in cooperation with the Bureau of Entomology.

In any consideration of the control of mosaic under practical growing conditions two phases of the question present themselves. One is

concerned with the origin of the disease in the seed bed; the other with the origin and spread of the disease in the field. Observations at Arlington, Va., during the season of 1912, indicate that if suitable insect carriers are active, mosaic plants in a field serve as a source of infection to all surrounding healthy plants. Should the origin of these diseased plants trace back directly to the seed bed, it is plain that any means of keeping mosaic infection from seed beds is at the same time a most important means of controlling the introduction and possible spread of the disease in the field. Should the disease originate directly in the field, the matter of successful control would be very difficult.

In various field experiments different investigators have shown conclusively that laborers in the practical operations of worming, suckering, and topping tobacco plants readily communicate infection from diseased to healthy plants. For this reason it is evident that the prompt removal of all mosaic plants is a most important means of preventing the spread of the disease in the field by human agencies. Since insect agencies may also act as carriers of infection in the field, the early removal of mosaic plants serves as an important check to the spread of the disease from this source. In whatever manner infection may be carried to healthy plants, it is obvious that mosaic plants must always be considered infection centers so long as they are allowed to remain in a field.

NATURE OF THE DISEASE.

Various theories have been advanced to explain the primary origin of the mosaic disease of tobacco. The view most generally accepted defines the disease as a disturbance of the enzymatic equilibrium induced by unfavorable conditions of growth. An enzymatic disease, is physiological in its nature, has it origin within the protoplasmic complex, and results in a serious and sometimes permanent impairment of the assimilative functions.

Although it has been shown by previous workers that the oxidase and peroxidase content of mosaic leaves is higher than in normal, healthy plants, this fact alone does not warrant its being considered the initial cause of the disease, for it might well be an effect rather than a cause. If it be considered that oxidases play an essential part in the metabolism of the plant, it would seem reasonable to suppose that the living cells suffering from lack of nutrition as a result of the disease in question would be stimulated to secrete an increased quantity of these enzyms, for it is a well-recognized physiological principle that cells suffering from lack of nourishment develop an increased amount of enzyms. It has been shown in the Office of Tobacco and Plant-Nutrition Investigation, for example, that during

the process of curing, the diastatic activity of tobacco leaves is greatly increased, since under these conditions the leaf cells are undergoing gradual starvation.

As a matter of fact, Bunzel,[1] from a biochemical study of the curly-top of sugar beets, a malady in many respects similar to the mosaic disease of tobacco, has recently shown that an abnormally high oxidase content is not necessarily a function of the disease, since his investigations show that plants subjected to various abnormal growing conditions, such as drought, overwatering, etc., develop a higher oxidase content than normal plants growing under favorable conditions. It is apparent, then, that a high oxidase content simply indicates disturbed metabolism arising from any one of a variety of unrelated causes.

Although purely physiological mosaic symptoms attended by chlorosis and various morphological changes in the leaves have been induced in tobacco plants by submitting them to sudden temperature changes, by severely cutting them back, etc., such disturbances are never infectious and do not usually persist under favorable conditions of growth. In the light of recent facts brought out in our experiments it is hard to see how the mosaic disease of tobacco can be logically placed in the category of purely physiological disturbances. The theory of parasitism accounts for the primary origin of the disease more consistently than the enzymatic hypothesis.

SUMMARY.

The mosaic disease of tobacco is communicable to a great variety of solanaceous plants. A number of species appear to be immune to the disease. In the susceptible group some species show a far greater degree of resistance to infection than others. The disease does not seem to be communicable to plants of other families. Although quite similar in many respects, the mosaic disease of pokeweed appears to be distinct from the mosaic disease of tobacco. Both diseases are readily communicable to plants of their kind.

The incubation period of the mosaic disease is variable, depending upon conditions favorable or unfavorable to the growth of the plants. The mosaic virus permeates all parts of the plant, including the roots and corollas as well as the foliage. The virus does not infect the embryos of seed produced by mosaic mother plants; therefore, such seeds produce healthy plants.

By artificial inoculation the mosaic disease can be induced to manifest itself, at first locally. In the course of time, however, the disease always becomes generalized.

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[1] Bunzel, H. H. Biochemical study of the curly-top of sugar beets. U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin 277, 28 p., 1913.

The visible expression of the disease is only an incidental feature of obscure pathogenic conditions within the plant and is considerably modified by external conditions.

Scions of *Nicotiana glauca* grafted upon mosaic stocks of *N. tabacum* show that the sap of *N. glauca* carries the virus even though this species does not manifest visible symptoms. *Solanum carolinense* frequently shows very slight visible symptoms. The character and intensity of the symptoms depend considerably upon the morphological structure of the leaf. Symptoms are more marked in thin, tender-leaved plants.

The mosaic disease, once initiated, becomes a progressive, incurable malady. Although many individuals escape the disease from a single inoculation, no plant of a susceptible species appears to be permanently immune.

The sap of mosaic plants after passing through a filter still retains its infectious properties. Mosaic material ground and dried retained its virulence 1½ years. The virus preserved by ether, toluene, and glycerin was virulent four months later, as was also the original juice, which had been allowed to undergo natural fermentation during this time.

Certain species of aphides are active disseminators of the mosaic disease. Not all sucking insects appear to act as carriers of infection. In greenhouses, where the disease is frequently very troublesome, the writer has kept it under complete control by frequent fumigations with nicotine to exclude aphides.

Observations in the field have shown that aphides of certain species may sometimes be responsible for the occurrence of the disease in the seed bed and its subsequent spread in the field.

Methods of controlling the mosaic disease of tobacco by fumigation have not been attempted for practical growing conditions. If the disease originates in the field after the plants have been transplanted, its practical control will be exceedingly difficult.

The simple processes of cutting back, frequent repotting, or otherwise subjecting plants to unfavorable conditions of growth are not sufficient to produce infectious mosaic symptoms. All facts indicate that the development of the disease depends entirely upon specific infection. The disease is not in any sense an air-borne contagion.

Although unbalanced enzymatic activities and physiological toxins have been considered responsible for the origin of the disease, these theories do not harmonize with all the facts at hand. Although organisms responsible for the mosaic disease have not yet been isolated, parasitism offers the more plausible explanation of the ultimate cause of the disease.

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